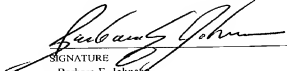


Form PTO 1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV 10-95) TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER 702-011069
		097/889938 <small>U.S. APPLICATION NO. 097/889938 (CFR 1.5)</small>
INTERNATIONAL APPLICATION NO PCT/EP00/00609	INTERNATIONAL FILING DATE 26.01.00 (26 January 2000)	PRIORITY DATES CLAIMED 27.01.99 (27 January 1999)
TITLE OF INVENTION METHOD FOR CONVEYING BNYVV RESISTANCE TO SUGAR BEET PLANTS		
APPLICANT(S) FOR DO/EO/US Kenneth RICHARDS, Gérard JONARD, Hubert GUILLEY and Cornelis M. P. VAN DUN		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information</p> <ol style="list-style-type: none"> <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371 <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371 <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1) <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). <input checked="" type="checkbox"/> has been transmitted by the International Bureau <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US) <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)) <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau) <input type="checkbox"/> have been transmitted by the International Bureau. <input type="checkbox"/> have not been made, however, the time limit for making such amendments has NOT expired <input checked="" type="checkbox"/> have not been made and will not be made. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)) <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). <p>Items 11. to 16. below concern document(s) or information included:</p> <ol style="list-style-type: none"> <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98 <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. <input checked="" type="checkbox"/> A FIRST preliminary amendment <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment <input type="checkbox"/> A substitute specification <input type="checkbox"/> A change of power of attorney and/or address letter <input checked="" type="checkbox"/> Other items or information <ol style="list-style-type: none"> WO 00/44915-Front Page with Abstract, specification, claims, drawings and sequence listing (30 pp.) International Preliminary Examination Report with Annexes (9 pp.) 		

JC17 Rec'd PCT/PTO 25 JUL 2001

U.S. APPLICATION NO. 09/889938		INTERNATIONAL APPLICATION NO. PCT/EP00/00609		ATTORNEY'S DOCKET NUMBER 702-011069	
17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO \$860.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) \$690.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) \$710.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$1000.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$100.00				CALCULATIONS PTO USE ONLY	
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$ 860.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e))				\$ 130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	32 - 20	12	X \$18.00	\$ 216.00	
Independent claims	8 - 3 =	5	X \$80.00	\$ 400.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				\$ 1606.00	
Reduction of 1/2 for filing by small entity, if applicable				\$ 0.00	
SUBTOTAL =				\$ 1606.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)) +				\$ 0.00	
TOTAL NATIONAL FEE =				\$ 1606.00	
Fee for recording the enclosed assignment (37 CFR 1.21(b)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) \$40.00 per property +				\$ 0.00	
TOTAL FEES ENCLOSED =				\$ 1606.00	
				Amount to be: Refunded	\$
				Charged	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>1606.00</u> to cover the above fees is enclosed b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees A duplicate copy of this sheet is enclosed c. <input checked="" type="checkbox"/> The Assistant Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>23-0650</u> . A duplicate copy of this sheet is enclosed NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status. SEND ALL CORRESPONDENCE TO Barbara E. Johnson 700 Koppers Building 436 Seventh Avenue Pittsburgh, Pennsylvania 15219-1818 Telephone: (412) 471-8815 Facsimile: (412) 471-4094 <div style="text-align: right;">  SIGNATURE Barbara E. Johnson NAME 31198 REGISTRATION NUMBER </div>					

09884938.121101

09/889938

JC17 Rec'd PCT/PTO 25 JUL 2001

PATENT APPLICATION/PCT

Atty. Docket No. 702-011069

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	
Kenneth RICHARDS	:	METHOD FOR CONVEYING BNYVV
Gérard JONARD	:	RESISTANCE TO SUGAR BEET
Hubert GUILLEY	:	PLANTS
Cornelis M. P. VAN DUN	:	
International Application	:	
No. PCT/EP00/00609	:	
International Filing Date	:	
26 January 2000	:	
Priority Date Claimed	:	
27 January 1999	:	
Serial No. Not Yet Assigned	:	
Filed Concurrently Herewith	:	

Pittsburgh Pennsylvania
July 26, 2001

PRELIMINARY AMENDMENT

BOX PCT
Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to initial examination, please amend the above-identified patent application as follows:

IN THE SPECIFICATION:

Please insert section headings and amend specification paragraphs as follows. (Pursuant to 37 CFR 1.121, marked-up versions of the amended specification paragraphs are attached.)

On page 1, after the title, please insert the following section headings.

BACKGROUND OF THE INVENTION

1. Field of the Invention

On page 1, after the first complete paragraph, please insert the following section heading.

2. Description of the Related Art

On page 3, after the fourth complete paragraph, please insert the following section heading.

SUMMARY OF THE INVENTION

Please delete the paragraph bridging pages 4 and 5 and insert the following replacement paragraph:

The homology or "degree of similarity" is used to denote nucleotide sequences which when aligned have similar (identical or conservatively replaced) nucleotides in like positions or regions. For example, two nucleotide sequences with at least 85% homology to each other have at least 85% homologous (identical or conservatively replaced nucleotides) in a like position when aligned optimally allowing for up to 3 gaps, with the provision that in respect of the gaps a total of not more than 15 amino acid residues is affected. The degree of similarity may be determined using methods well known in the art (see, for example, Wilbur, W.J. and Lipman, D.J. "Rapid Similarity Searches of Nucleic Acid and Protein Data Banks." Proceedings of the National Academy of Sciences USA 80, 726-730 (1983) and Myers E. and Miller W. "Optimal Alignments in Linear Space". Comput. Appl. Biosci. 4:11-17(1988)). One programme which may be used in determining the degree of similarity is the MegAlign Lipman-Pearson one pair method (using default parameters) which can be obtained from

DNASTar Inc, 1228, Selfpark Street, Madison, Wisconsin, 53715, USA as part of the Lasergene system. The test for homology of the sequence is based on the percent identity which is calculated by Fast DB based on the following parameters: mismatch penalty 1.0, gap penalty (1.00), gap size penalty 0.33 and joining penalty 30.0.

After the paragraph bridging pages 4 and 5, please insert the following section headings and specification paragraph:

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagrammatic representation of the genomic organization of Beet Necrotic Yellow Vein Virus;

Fig. 2 is a diagram of the physical maps of pVDH239 and pVDH240;

Fig. 3 is a depiction of Southern blot analysis of T-DNA insertions into the genome of the primary sugar beet transformant T157-01;

Fig. 4 is a diagram of the individual ELISA values of the root extracts of various populations of sugar beet plants;

Fig. 5A is a graph of Rhizomania resistance of T157-01 F1; and

Fig. 5B is a depiction of Southern blot analysis of T-DNA insertions into the genome of the F1 progeny plants of T157-01.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

On page 10, please delete the last complete paragraph, and insert the following replacement paragraph:

Figure 4 shows diagrams of the individual ELISA values of the root extracts of sugar beet plants of the populations CaduX (susceptible control), Rifle (rhizomania tolerant variety), Rhizor (rhizomania tolerant variety) and T157-01 (GUS-positive F1 individuals) after inoculation with BNYVV-infested soil. Each number at the horizontal axis represents an individual plant.

IN THE CLAIMS:

Original claims 1-29 were amended during Chapter II proceedings by substituting new claims 1-29 in a letter dated February 4, 2001. Please cancel original claims 1-29 and cancel amended claims 1-29 and rewrite them as new claims 30-61 as follows:

30. A method for conveying resistance to beet necrotic yellow vein virus (BNYVV) to a sugar beet plant, comprising the following steps:

- (a) preparing a DNA fragment of at least 15 nucleotides in a sequence that is at least 70% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of the beet necrotic yellow vein virus (BNYVV);
- (b) introducing said DNA fragment, operatively linked to a promoter that is active in sugar beet plants, into a sugar beet plant cell to obtain a transformed sugar beet cell; and
- (c) regenerating a transgenic sugar beet plant from the transformed sugar beet plant cell.

31. The method as claimed in claim 30, wherein the DNA fragment is at least 80% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of said virus.

32. The method according to claim 30, wherein the fragment has a nucleic acid sequence that corresponds with the homology indicated in claim 30 to nucleotides 153 to 3258 of RNA 1 of said virus.

33. The method according to claim 30, wherein the fragment has a nucleic acid sequence that corresponds with the homology indicated in claim 30 to nucleotides 169 to 539 of RNA 1 of said virus.

34. The method according to claim 30, wherein the fragment has a nucleic acid sequence that corresponds with the homology indicated in claim 30 to nucleotides 1226 to 1683 of RNA 1 of said virus.

35. The method according to claim 30, wherein the fragment has a nucleic acid sequence that corresponds with the homology indicated in claim 30 to nucleotides 2754 to 3192 of RNA 1 of said virus.

36. The method according to claim 30, wherein the fragment consists of 6746 nucleotides.

37. The method as claimed in claim 30, wherein the fragment is introduced into the cell by means of a DNA vector harboring the fragment and transcription and translation regulatory sequences operably linked therewith.

38. A transformation vector for conveying resistance to BNYVV to a plant, harboring a fragment of at least 15 nucleotides in a sequence that is at least 70% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of said virus, and transcription and translation regulatory sequences operably linked therewith.

39. The vector as claimed in claim 38, wherein the fragment is at least 80% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of said virus.

40. The vector according to claim 38, wherein the fragment has a nucleic acid sequence that corresponds with the homology indicated in claim 38 to nucleotides 153 to 3258 of RNA 1 of said virus.

41. The vector according to claim 38, wherein the fragment has a nucleic acid sequence that corresponds with the homology indicated in claim 38 to nucleotides 169 to 539 of RNA 1 of said virus.

42. The vector according to claim 38, wherein the fragment has a nucleic acid sequence that corresponds with the homology indicated in claim 38 to nucleotides 1226 to 1683 of RNA 1 of said virus.

43. The vector according to claim 38, wherein the fragment has a nucleic acid sequence that corresponds with the homology indicated in claim 38 to nucleotides 2754 to 3192 of RNA 1 of said virus.

44. The vector according to claim 38, wherein the fragment consists of 6746 nucleotides.

45. A plant cell, exhibiting a resistance to BNYVV, comprising in its genome a DNA fragment of at least 15 nucleotides in a sequence which is at least 70% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of said virus.

46. The plant cell as claimed in claim 45, wherein the fragment is at least 80% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of said virus.

47. The plant cell according to claim 45, wherein the fragment has a nucleic acid sequence that corresponds with the homology indicated in claim 45 to nucleotides 153 to 3258 of RNA 1 of said virus.

48. The plant cell according to claim 45, wherein the fragment has a nucleic acid sequence that corresponds with the homology indicated in claim 45 to nucleotides 169 to 539 of RNA 1 of said virus.

49. The plant cell according to claim 45, wherein the fragment has a nucleic acid sequence that corresponds with the homology indicated in claim 45 to nucleotides 1226 to 1683 of RNA 1 of said virus.

50. The plant cell according to claim 45, wherein the fragment has a nucleic acid sequence that corresponds with the homology indicated in claim 45 to nucleotides 2754 to 3192 of RNA 1 of said virus.

51. The plant cell according to claim 45, wherein the fragment consists of 6746 nucleotides.

52. The plant cell as claimed in claim 45 being part of a sugar beet plant that is resistant against BNYVV.

53. A method for the production of a sugar beet plant that is resistant against BNYVV comprising regeneration from a plant cell as claimed in claim 45.

54. A sugar beet plant, exhibiting a resistance to BNYVV, consisting at least partly of plant cells as claimed in claim 45.

55. A progeny of sugar beet plant as claimed in claim 54.

56. Seeds of a sugar beet plant as claimed in claim 54.

57. Vegetatively reproducible structures from a plant according to claim 54.

58. The method as claimed in claim 30, wherein the DNA fragment is at least 90% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of said virus.

59. The method as claimed in claim 30, wherein the DNA fragment is at least 95% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of said virus.

60. The vector as claimed in claim 38, wherein the fragment is at least 90% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of said virus.

61. The vector as claimed in claim 38, wherein the fragment is at least 95% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of said virus.

IN THE ABSTRACT:

After the claims, please insert a page containing the Abstract Of The Disclosure, which is attached hereto as a separately typed page.

REMARKS

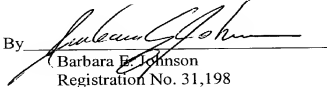
The specification has been amended to place the application in conformance with standard United States patent practice.

Examination and allowance of pending claims 30-61 are respectfully
requested.

Respectfully submitted,

WEBB ZIESENHEIM LOGSDON
ORKIN & HANSON, P.C.

By

A handwritten signature in black ink, appearing to read "Barbara E. Johnson", is written over a horizontal line.

Barbara E. Johnson
Registration No. 31,198
Attorney for Applicants
700 Koppers Building
436 Seventh Avenue
Pittsburgh, PA 15219-1818
Telephone: 412-471-8815
Facsimile: 412-471-4094

MARKED-UP AMENDED SPECIFICATION PARAGRAPHS

Paragraph bridging pages 4 and 5

The homology or "degree of similarity" is used to denote nucleotide sequences which when aligned have similar (identical or conservatively replaced) nucleotides in like positions or regions. For example, two nucleotide sequences with at least 85% homology to each other have at least 85% homologous (identical or conservatively replaced nucleotides) in a like position when aligned optimally allowing for up to 3 gaps, with the [provison] provision that in respect of the gaps a total of not more than 15 amino acid [resides] residues is affected. The degree of similarity may be determined using methods well known in the art (see, for example, Wilbur, W.J. and Lipman, D.J. "Rapid Similarity Searches of Nucleic Acid and Protein Data Banks." Proceedings of the National Academy of Sciences USA 80, 726-730 (1983) and Myers E. and Miller W. "Optimal Alignments in Linear Space". Comput. Appl. Biosci. 4:11-17(1988)). One programme which may be used in determining the degree of similarity is the MegAlign Lipman-Pearson one pair method (using default parameters) which can be obtained from DNASTar Inc, 1228, Selfpark Street, Madison, Wisconsin, 53715, USA as part of the Lasergene system. The test for homology of the sequence is based on the percent identity which is calculated by Fast DB based on the following parameters: mismatch penalty 1.0, gap penalty (1.00), gap size penalty 0.33 and joining penalty 30.0.

Page 10, last complete paragraph

Figure 4 shows diagrams of the individual ELISA values of the root extracts of sugar beet plants of the populations Cadyx [(susceptibel] (susceptible) control), Rifle (rhizomania tolerant variety), Rhizor (rhizomania tolerant variety) and

T157-01 (GUS-positive F1 individuals) after inoculation with BNYVV-infested soil.

Each number at the horizontal axis represents an individual plant.

METHOD FOR CONVEYING BNYVV
RESISTANCE TO SUGAR BEET PLANTS

ABSTRACT OF THE INVENTION

The present invention relates to a method for conveying resistance to beet necrotic yellow vein virus (BNYVV) to a sugar beet plant, which method comprises the following steps: (a) preparing a DNA fragment of at least 15 nucleotides in a sequence that is essentially homologous to the corresponding nucleotide sequence of the genomic RNA 1 of the beet necrotic yellow vein virus (BNYVV); (b) introducing said DNA fragment, operatively linked to a promotor that is active in sugar beet plants, into a sugar beet plant cell to obtain a transformed sugar beet cell; and (c) regenerating a transgenic sugar beet plant from the transformed sugar beet plant cell.

7/ PATS

098899 09/889938

JC17 Rec'd PCT/PTO 25 JUL 2001

WO 00/44915

PCT/EP00/00609

METHOD FOR CONVEYING BNYVV RESISTANCE TO
SUGAR BEET PLANTS

The present invention relates to a method for conveying viral resistance to beet necrotic yellow vein virus (BNYVV) to a sugar beet plant. Furthermore, the invention relates to virus-resistant plants obtained according to this method, as well as to seeds and progeny derived therefrom.

Plant viruses are a serious problem for many of the major agricultural crops. Thus, BNYVV which is transmitted by the soilborne plasmodiophoromycete fungus Polymyxa betae, is the cause of the economically important disease of sugar beet (Beta vulgaris), known as rhizomania. This disease was first reported in Italy in the 1950s and has since become a major threat to sugar beet crops in most growing areas in the world.

Under field conditions BNYVV is generally confined to the subterranean portions of the plant. Infected sugar beet roots exhibit an increased proliferation and necrosis of the lateral roots, a decrease in the total mass of the main root and a consequent reduction in sugar yield. Plant death may also follow infection, particularly if the crop is infected early in season. Occasionally, the virus will ascend into the leaves where it can provide invasive vein-associated chlorotic and necrotic lesions.

BNYVV is a type member of the benyvirus family, which are fungus-transmitted, rod-shaped viruses possessing a divided genome composed of two or more components of single-stranded (ss) RNA. Four different plus sense ssRNA species of BNYVV have been identified, which are referred to as RNAs 1 to 4 in decreasing order of size (RNA 1: 6.8 kb; RNA 2: 4.7 kb; RNA 3: 1.8 kb; RNA 4: 1.5 kb). Some Japanese isolates also contain a fifth RNA component (RNA 5: 1.45 kb). All RNAs have been cloned and sequenced. The genetic map of the viral RNAs is shown in figure 1.

All five RNAs terminate in 3' poly-A tails, and RNAs 1-4 have cap structures at their 5' termini. RNAs 1

and 2 encode basic host-independent "housekeeping" functions, while the smaller RNAs intervene specifically in the natural infection process, including vector-mediated infection of sugar beet roots, proliferation within the root system and production of rhizomania symptoms. Thus, RNA 1 has been shown to code for viral RNA polymerase activity and RNA 2 encodes the 21-kd viral coat-protein. The 3'-proximal half of RNA 2 carries a group of three successive slightly overlapping viral genes, known as the triple gene block (TGB3), that are closely similar to a cluster of three genes in other rod-shaped plant viruses, and are involved in cell to cell movement of the virus. RNA 3 is associated with massive proliferation of fine rootlets in sugar beet and facilitates the spread of the virus in root tissue, whereas RNA 4 increases the efficiency of transmission of the virus by the fungus.

Fungus-transmitted viruses, such as BNYVV may be retained in resting spores in soil for years once a field becomes infested. As there exist no effective chemical or physical methods for eliminating the virus, neither in the plants nor in the soil, the only option for the sugar beet farmer is the use of genetically resistant cultivars. Several companies have provided a number of tolerant, even partially resistant varieties, by transferring wild type tolerance genes into commercially varieties through breeding. This is, however, a very tedious and time-consuming process, generally taking a long time before useful resistant plants are obtained. In addition, under high disease pressure tolerant or partially resistant plants will develop disease symptoms because the resistance level is too low. Another problem with tolerant or only partially resistant plants lies in the fact that virus populations continue to grow resulting in the possible emergence of a BNYVV strain which may break the resistance/tolerance genes.

The rapid revolution in the areas of plant engineering has led to the development of new strategies to confer genetic resistance to viruses. Resistance to viral diseases through the introduction of portions of viral genome sequences has become a new source of resistance. The viral sequence (construct) is transformed into a plant by combining the use of appropriate cell or tissue culture techniques and a DNA delivery system such as the well known Agrobacterium tumefaciens transformation system or the direct DNA transfer mediated by chemicals like polyethylene glycol (PEG).

Known methods of inducing pathogen defense mechanisms in plants are for example described in EP 9687106.0, which relates to a method for inducing resistance to a virus comprising a TGB3 sequence. The publication describes that it was possible to induce BNYVV resistance by a method, which consists of transforming plant cells with a DNA construct corresponding to a fragment of the nucleic acid sequence of the genomic or subgenomic RNA 2 of the BNYVV. The disadvantage of this method is however, that only tolerance is obtained, and not resistance. In tolerant host plants there can still be normal levels of plant virus replication, but the plant shows little or no visible signs of infection, whereas in resistant hosts the virus replication is low or even absent.

In WO 93/25068 viral resistance in plants is induced by plant transformation with a replicase portion (RNA 1) of a plant virus genome. This publication does not refer to sugar beet, nor to BNYVV.

Sugar beet are known to be recalcitrant species in genetic engineering, complicating successful induction of BNYVV resistance. However, because of the extent and impact of the viral disease of BNYVV there is a major interest to improve the sources of genetic resistance in sugar beet plants.

The object of the present invention is, therefore, to provide means to obtain sugar beet plants,

that exhibit resistance to BNYVV. Preferably, they exhibit total resistance or immunity, by combining different approaches and by using methods which do not allow the virus to replicate.

5 This is achieved by the invention by providing a method for conveying resistance to beet necrotic yellow vein virus (BNYVV) to a sugar beet plant, comprising the following steps:

(a) preparing a DNA fragment of at least 15
10 nucleotides in a sequence that is essentially homologous to the corresponding nucleotide sequence of the genomic RNA 1 of the beet necrotic yellow vein virus (BNYVV),

(b) introducing said DNA fragment, operately linked to a promotor that is active in sugar beet plants,
15 into a sugar beet plant cell to obtain a transformed sugar beet cell; and

(c) regenerating a transgenic sugar beet plant from the transformed sugar beet plant cell.

Thus, a sugar beet plant is obtained which
20 exhibits a durable resistance and in which the virus does not replicate. This is a unique aspect of the transformant of the invention and has not been disclosed before.

According to the invention the essential
25 sequence homology of the fragment is a homology of at least 70%, preferably at least 80%, more preferably at least 90%, most preferably at least 95%.

The homology or "degree of similarity" is used to denote nucleotide sequences which when aligned have
30 similar (identical or conservatively replaced) nucleotides in like positions or regions. For example, two nucleotide sequences with at least 85% homology to each other have at least 85% homologous (identical or conservatively replaced nucleotides) in a like position
35 when aligned optimally allowing for up to 3 gaps, with the provision that in respect of the gaps a total of not more than 15 amino acid residues is affected. The degree of similarity may be determined using methods well known

in the art (see, for example, Wilbur, W.J. and Lipman, D.J. "Rapid Similarity Searches of Nucleic Acid and Protein Data Banks." Proceedings of the National Academy of Sciences USA 80, 726-730 (1983) and Myers E. and Miller W. "Optimal Alignments in Linear Space". Comput. Appl. Biosci. 4:11-17(1988)). One programme which may be used in determining the degree of similarity is the MegAlign Lipman-Pearson one pair method (using default parameters) which can be obtained from DNASTar Inc, 1228, Selfpark Street, Madison, Wisconsin, 53715, USA as part of the Lasergene system. The test for homology of the sequence is based on the percent identity which is calculated by Fast DB based on the following parameters: mismatch penalty 1.0, gap penalty (1.00), gap size penalty 0.33 and joining penalty 30.0.

Preferred embodiments of the invention use various fragments having nucleic acid sequences that correspond with the homology indicated or completely to nucleotides 153 to 3258, 169 to 539, 1226 to 1683, 2754 to 3192 or to all of the 6746 nucleotides of RNA 1.

The present invention also includes DNA which hybridises to the DNA of the present invention and which codes for RNA1. Preferably, such hybridisation occurs at, or between, low and high stringency conditions. In general terms, low stringency conditions can be defined as 3 x SCC at about ambient temperature to about 65°C, and high stringency conditions as 0.1 x SSC at about 65°C. SSC is the name of a buffer of 0.15M NaCl, 0.015M trisodium citrate. 3 x SSC is three times as strong as SSC and so on.

The fragment can be introduced into a regenerable plant cell by means of a DNA vector harboring the fragment and transcription and translation regulatory sequences operably linked therewith using standard plant transformation methods such as Agrobacterium-mediated transformation of cells embedded in plant tissues such as cotyledons (Krens et al., Plant Science 116: 97-106; 1996) or polyethylene glycol-mediated DNA uptake of

single cells like the guard cell protoplasts (Hall et al., Nature Biotechnology 14: 1133-1138; 1996). The DNA vector harboring the fragment is also part of the present invention.

5 The use of constructs constructed such that the gene sequence inhibits or promotes gene expression is quite well understood. A complete gene sequence, under the control of a promoter that operates effectively in the plant, will generally overexpress the gene product, leading to an amplification of the effect of the protein so produced. Sometimes the gene product is reduced: this phenomenon is termed "co-suppression". Downregulating this gene can be done by several techniques. It can be done by 'dominant-negative' constructs. These contain the specific DNA binding domain as well as possible dimerisation domains but are transcriptionally inactive. They 'sit' on the promoters of the target genes and thereby prevent the binding of the endogenous protein. Additionally, reduction of the gene product can also be obtained by using such dominant negative mutation, or by reversing the orientation of the gene sequence with respect to the promoter so that it produces a type of gene product called "antisense" messenger RNA.

A DNA construct according to the invention may be an "antisense" construct generating "antisense" RNA or a "sense" construct (encoding at least part of the functional protein) generating "sense" RNA.

"Antisense RNA" is an RNA sequence which is complementary to a sequence of bases in the corresponding mRNA: complementary in the sense that each base (or the majority of bases) in the antisense sequence (read in the 3' to 5' sense) is capable of pairing with the corresponding base (G with C, A with U) in the mRNA sequence read in the 5' to 3' sense. Such antisense RNA may be produced in the cell by transformation with an appropriate DNA construct arranged to generate a transcript with at least part of its sequence complementary to at least part of the coding strand of

the relevant gene (or of a DNA sequence showing substantial homology therewith).

"Sense RNA" is an RNA sequence, which is substantially homologous to at least part of the corresponding mRNA sequence. Such sense RNA may be produced in the cell by transformation with an appropriate DNA construct arranged in the normal orientation so as to generate a transcript with a sequence identical to at least part of the coding strand of the relevant gene (or of a DNA sequence showing substantial homology therewith). Suitable sense constructs may be used to inhibit gene expression (as described in International Patent Publication WO91/08299).

DNA constructs according to the invention may comprise a base sequence at least 10 bases (preferably at least 35 bases) in length for transcription into RNA. There is no theoretical upper limit to the base sequence - it may be as long as the relevant mRNA produced by the cell - but for convenience it will generally be found suitable to use sequences between 100 and 1000 bases in length. The preparation of such constructs is described in more detail below.

As a source of the DNA base sequence for transcription, a suitable cDNA or genomic DNA, RNA or synthetic polynucleotide may be used. The transcriptional initiation region (or promoter) operative in plants may be a constitutive promoter (such as the 35S cauliflower mosaic virus promoter) or an inducible or developmentally regulated promoter, as circumstances require. Suitable DNA sequences for control of expression of the plant expressible genes (including marker genes), such as transcriptional initiation regions, enhancers, leader sequences, non-transcribed leaders and the like, may be derived from any gene that is expressible in a plant cell. Also hybrid promoters combining functional portions of various promoters, or synthetic equivalents thereof can be employed. Apart from constitutive promoters,

inducible promoters, or promoters otherwise regulated in their expression pattern, e.g. developmentally or cell-type specific, may be used to control expression of the expressible genes according to the invention. For
5 example, it may be desirable to modify protein activity at certain stages of the plant's development. Use of a constitutive promoter will tend to affect protein levels and functions in all parts of the plant, while use of a tissue-specific promoter allows more selective control of
10 gene expression and affected functions.

Another option under this invention is to use inducible promoters. Promoters are known which are inducible by pathogens, by stress, by chemicals and by environmental signals. The induction of the gene activity
15 by internal or external induction is within the scope of the present invention. Promoters of this type enable the inducibility of the gene activity in a controlled manner, thus the plant can develop normally without any undue influence by the transgene gene. Promoters that are
20 inducible promoters include those described in DE 4446342 (fungus and auxin inducible PRP-1), WO 96/28561 (fungus inducible PRP-1), EP 0 712 273 (nematode inducible), EP 0 330 479 and US 5,510,474 (stress inducible), WO/96/12814 (cold inducible), and Zeneca's alcohol inducible
25 promoter. Other inducible promoters are described in EP 0 494 724, EP 0 619 844, WO 92/19724. Thus the gene product, whether antisense or sense RNA or the peptide, is only produced in the tissue at the time when its action is required.

30 As mentioned above, the term "inducible promoter" includes promoters which may be induced chemically. The use of a promoter sequence which is controlled by the application of an external chemical stimulus is most especially preferred. The external
35 chemical stimulus is preferably an agriculturally acceptable chemical, the use of which is compatible with agricultural practice and is not detrimental to plants or mammals. The inducible promoter region most preferably

comprises an inducible switch promoter system such as, for example, a two component system such as the *alcaA/alcR* gene switch promoter system described in the published International Publication No. WO 93/21334, the *ecdysone* switch system as described in the International Publication No. WO 96/37609 or the *GST* promoter as described in published International Patent Application Nos. WO 90/08826 and WO 93/031294, the teachings of which are incorporated herein by reference. Such promoter systems are herein referred to as "switch promoters". The switch chemicals used in conjunction with the switch promoters are agriculturally acceptable chemicals making this system particularly useful in the method of the present invention.

The skilled person will be capable of selecting a DNA vector for use in these methods. An example of a suitable vector for the *Agrobacterium*-mediated transformation is *pBIN19*. Suitable vectors for the PEG-mediated transformation include the *pBluescript* vector or *pIGPD7* (Hall et al., *Nature Biotechnology* 14: 1133-1138; 1996). Introduction of the fragment into these vectors can be achieved by means of standard molecular biological techniques as for example described in Sambrook et al., "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Laboratory Press; 1989.

Transformed plants obtained by the method of the present invention show absolute resistance, or immunity, to *BNYVV*. In contrast, previous attempts to convey resistance to plants to *BNYVV* (Kallerhof et al., *Plant Cell Reports* 9: 224-228, 1990; and Mannerlof et al., *Euphytica* 90: 293-299, 1996) or other viruses, such as tobacco mosaic virus (*TMV*) (Donson et al., *Mol. Plant-Microbe Interact.* 6: 635-642; 1993) by transforming plants with portions of the viral genome were less successful. Inoculated leafs still showed symptoms of infection, thus indicating that the resistance is not absolute. It is therefore surprising that the method of

the invention is capable of conveying absolute resistance to BNYVV to sugarbeet plants.

Furthermore the invention relates to a transformed plant cell and a transgenic plant resistant to BNYVV as well as reproducible structures, such as seeds, calluses, buds, embryos, obtained from the transgenic plants and the progeny derived therefrom.

In a preferred embodiment of the invention the resistance described herein can be combined with other types of resistance or tolerance to BNYVV.

The invention will further be illustrated in the following examples and figures, but is not limited thereto.

Figure 1 shows a diagrammatic representation of the genomic organisation of Beet Necrotic Yellow Vein Virus (based on Jupin et al., Seminars in Virology vol 2.2: 112-129; 1991).

Figure 2 shows the physical maps of pVDH239 and pVDH240. LB=left border, RB=right border, P35S=CaMV 35S promoter, NPTII= neomycin phosphotransferase II, T35S=CaMV 35S polyadenylation signal, GUSINT=beta-glucuronidase gene, BNYVVPolTRUNC=BNYVV cDNA1 fragment, Tnos=nopaline synthase gene derived polyadenylation signal. The positions of the main restriction enzyme recognition sites are indicated.

Figure 3 shows a Southern blot analysis by which the number of T-DNA insertions integrated into the genome of the primary sugar beet transformant T157-01 has been determined. The outline of the T-DNA structure of the binary vector pVDH239 is shown at the top.

Figure 4 shows diagrams of the individual ELISA values of the root extracts of sugar beet plants of the populations Caduex (susceptible control), Rifle (rhizomania tolerant variety), Rhizor (rhizomania tolerant variety) and T157-01 (GUS-positive F1 individuals) after inoculation with BNYVV-infested soil. Each number at the horizontal axis represents an individual plant.

Figure 5B shows a Southern blot analysis by which the number of T-DNA insertions integrated into the genome of the F1 progeny plants of T157-01 has been determined as well as a diagram of the individual ELISA values of the root extracts of the F1 progeny plants of T157-01) after inoculation with BNYVV-infested soil (figure 5A). The numbers on top of the Southern blots represent the lab-codes of the individual F1 progeny plants. The ELISA values indicated by "Genotype 1" in the lower pannel correspond to the individuals showing a single band in the Southern blot (2012, 2019, 2021, 2029, 2030, 2031, 2034, 2035, 2038, 2042, 2044, 2046, 2051, 2052, 2061, 2066, 2068, 2069), whereas the ELISA values indicated by "Genotype 2 + 3" in the lower pannel correspond to the individuals showing 2 or 3 bands in the Southern blot (1999, 2000, 2001, 2007, 2008, 2011, 2013, 2014, 2015, 2016, 2017, 2018, 2020, 2022, 2023, 2024, 2025, 2026, 2027, 2028, 2032, 2033, 2036, 2037, 2039, 2040, 2041, 2043, 2045, 2047, 2048, 2049, 2050, 2053, 2054, 2055, 2056, 2057, 2058, 2059, 2060, 2062, 2063, 2064, 2065, 2067, 2070). The ELISA values indicated by "GUS(-) segregants" in the lower pannel correspond to the individual F1 progeny plants which are GUS-negative (1997, 1998, 2002, 2004, 2005, 2006, 2009, rest not shown).

EXAMPLES

EXAMPLE 1

30 Preparation of construct with truncated BNYVV replicase sequence

Two primer combinations were used to obtain the cDNA clones of BNYVV for cloning in the transformation vector (Bouzoubaa et al. J. Gen. Virol. 68:615-626; 1987).

For the 5'-end the primers were

- P1: 5'-CGCGGATCCACCATGGCAGATTCGTTC-3' (containing a BamHI and NcoI restriction site and nucleotides identical to nucleotides 153-168), and
- P2: 5'-GACGAATTC AAGTCGCTTTTC-3' (EcoRI restriction site and nucleotides complementary to nucleotides 288-301). For the 3'-end the primers were
- P3: 5'-GACGAATTCGAAAGATGAGTCTA-3' (EcoRI site and nucleotides identical to nucleotides 2799-2812), and
- P4: 5'-CGCAGATCTTTAACTGCTCATCACAAC-3' (BglII site and 10 nucleotides complementary to nucleotides 3244-3258 and stop codon).

- Before cloning the fragments into the Bluescript vector (Stratagene, La Jolla, CA, USA) the SalI/HincII/AccI site was replaced by a BglII site. After 15 amplification of BNYVV cDNA using P1 and P2 a DNA fragment was obtained which was digested with BamHI and EcoRI and inserted into the modified, BamHI/EcoRI cleaved pBluescript vector to yield pKSNBP01. Subsequently, BNYVV cDNA1 was amplified using P3 and P4. The resulting 20 DNA fragment was cleaved by EcoRI and BglII and inserted into pKSNBP01 cleaved with EcoRI and BglII, yielding pKSNBP02.

- Thereafter the AccI fragment of BNYVV cDNA1 identical to nucleotides 250-2815 was cloned in AccI 25 cleaved pKSNBP02. Thus, a pKSNBP02trunc was obtained which comprised the BNYVV cDNA1 fragment (poltrunc) identical to the nucleotides 153-3258, flanked by a BamHI site at the 5'-end and by a BglII fragment at the 3'-end. The poltrunc fragment was cloned as BamHI-BglII fragment 30 in the BamHI site of pVDH4 such that a functional sense poltrunc fragment was placed behind the CaMV 35S promoter and before the nopaline terminator sequence. The complete construct, carrying the CaMV35S promoter, the poltrunc fragment and nopaline terminator sequence was excised 35 from the plasmid with ClaI and cloned in the binary transformation vector pVDH212 in which the BamHI site was converted into a ClaI site by inserting a molecular linker.

pVDH212 is a pBIN19 derived binary transformation vector, which contains 35S-NPTII (neomycin phosphotransferase under control of the CaMV 35S promotor as a selectable marker gene conferring resistance to 5 kanamycin), as well as 35S-GUSi (gene encoding beta-glucuronidase (Vancanneyt et al., Mol. Gen. Genet. 220: 245-250; 1990). Thus, two different transformation vectors containing the poltrunc construct were obtained: one, pVDH239, with the poltrunc construct in the same 10 transcriptional direction as the NPTII and GUS gene, and another, pVDH240, with the poltrunc construct having an opposite transcriptional direction. The binary transformation vectors pVDH239 and 240 (figure 2) were conjugated and transferred to Agrobacterium tumefaciens 15 LBA4404 (Phabagen Collection, Utrecht, the Netherlands) resulting in HAT1239 (for pVDH239) and HAT1240 (for pVDH240) strains. HAT1239 and HAT1240 were used for transformation of sugar beet.

20 EXAMPLE 2

Transformation of sugar beet

Methods for Agrobacterium-mediated transformation using binary vectors are well established 25 and known to a person skilled in the art.

To obtain transformed sugar beet plants, O-type B8M5.9 plants were transformed according to Krens et al. (Plant Science 116: 97-106; 1996). Transformation with Agrobacterium HAT1239 yielded 8 transformants: T156-03, 30 T156-06, T156-09, T156-10, T156-13, T156-20, T150-30, and T157-01, and with Agrobacterium HAT1240 12 transformants were obtained: T183-01, T183-02, T183-06, T183-10, T183-16, T183-19, T183-21, T183-23, T183-26, T184-02, T184-03 and T184-04. The transformants were induced to flowering 35 by vernalisation and used to produce seeds either through self-pollination (selfing or S1 seed) or cross-pollination on male sterile plants (hybrid or F1 seed). The pollination was performed in closed pollen chambers

to prevent pollination by sugar beet pollen from a different source.

The F1 seeds were used as starting material to carry out a bioassay for BNYVV resistance. The seedlings were screened initially for GUS activity which indicates the presence of the T-DNA. GUS-negative segregants were used as a negative control in the bioassay. Depending on the total number of T-DNA inserts, the resistance, if present, can segregate within the GUS-positive population. Only T157-01 derived progeny showed BNYVV resistance.

Subsequently, the primary transformant T157-01 was analysed in further detail on Southern blot. Genomic DNA was isolated from leaves, digested separately with EcoRI, BamHI and SacI and used to prepare a Southern blot which was subsequently probed with GUS. From this experiment it was concluded that the primary transformant contained three T-DNA inserts (figure 3).

20 **EXAMPLE 3**

Bioassay

A lateral root bioassay was developed as a single plant test for the selection of rhizomania resistant sugarbeet plants. One-week-old plantlets were transplanted in a standard mixture of 10% rhizomania infected soil and further incubated for 4 weeks. The conditions for infection were optimised and standardised on the level of the infection pressure and the growth of rootlets (only formation of hairy roots). Incubation conditions were: 18.9-19°C day/night, 70% relative humidity, 10.000 lux white light, pots at 32.5 cm from the lamps.

The amount of virus in the rootlets which was directly correlated with the resistance mechanism in the plant was measured by an ELISA method. The cut-off value for resistant plants was put at an OD-value of 0.2 (Clark et al. J. Virol. Meth. 15:213-222; 1987). Four weeks

after transplantation the lower part of the roots was used for ELISA. The root piece was dried on a filter paper and transferred to a mortar. Extraction buffer was added at a ratio of 10 volume equivalents of the root weight (dilution 1/10). Root juice was extracted by hand crushing. The juice was transferred to 1.5 ml tubes and centrifuged (300 rpm, 10 minutes) the supernatants were kept on ice and assayed. Populations tested were:

10	Population	Number of plants	Remarks
	T 157-01 (F1)	73	GUS(+) individuals
	Cadyx F 052	26	sensitive control
	Rhizor F 202	27	tolerant control
	Rifle	28	tolerant control

15

Within the group of T157-01 GUS(+) plants two categories can be observed (figure 4). One category displays immunity to BNYVV (ELISA cut-off 0.2) with an average ELISA value of 0.006 which is the background level of the experimental system. The other category shows normal susceptibility with an average ELISA value in the range of the susceptible control.

The infection pressure in this bioassay was high due to the increase of temperature during a part of the infection period. Consequently there was no clear distinction between Cadyx and Rizor/Rifle (figure 4). On the other hand, the resistant plants selected in the T 157-01 F1 progeny could be considered as totally resistant independent of the rhizomania infection pressure (figure 4). In conclusion, the introduced construct containing the BNYVV cDNA1 fragment resulted in a dramatic negative effect on the multiplication of BNYVV in the lateral roots of the inoculated transgenic sugar beet plants which effectively rendered the plants completely resistant to rhizomania.

In order to explain the segregation of the GUS(+) population in a resistant and a susceptible category the individual plants were analysed in a separate experiment for resistance to BNYVV and simultaneously for the presence of the T-DNAs by Southern analysis using SacI as restriction enzyme and GUS as a probe. The results, as depicted in Figure 5A and 5B, point out that the GUS(+) plants within this population which contained a single band on the Southern blot were all susceptible (average ELISA value 0.63), whereas the GUS(+) plants which contained either 2 or 3 bands on the Southern blot were all resistant (average ELISA value: 0.21). The GUS(-) segregants were all susceptible (average ELISA value 0.80). Apparently the resistant phenotype is linked to the presence of the upper and lower band of this particular band pattern obtained on Southern blot, whereas the presence of the middle band is not linked to the resistant phenotype. It is therefore concluded that the segregation of the GUS(+) population into a susceptible and a resistant category can be explained by the fact that one of the T-DNAs which can result in a GUS(+) phenotype does not contribute to the resistance to BNYVV.

CLAIMS

(38)

1. Method for conveying resistance to beet necrotic yellow vein virus (BNYVV) to a sugar beet plant, comprising the following steps:
- (a) preparing a DNA fragment of at least 15
5 nucleotides in a sequence that is at least 70% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of the beet necrotic yellow vein virus (BNYVV),
 - (b) introducing said DNA fragment, operately
linked to a promotor that is active in sugar beet plants,
10 into a sugar beet plant cell to obtain a transformed sugar beet cell; and
 - (c) regenerating a transgenic sugar beet plant from the transformed sugar beet plant cell.
2. Method as claimed in claim 1, wherein the
15 DNA fragment is at least 80%, preferably at least 90%, more preferably at least 95% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of said virus.
3. Method according to claim 1 or 2,
20 characterized in that the fragment has a nucleic acid sequence that corresponds with the homology indicated in claims 1 and 2 to nucleotides 153 to 3258 of RNA 1 of said virus.
4. Method according to claim 1 or 2,
25 characterized in that the fragment has a nucleic acid sequence that corresponds with the homology indicated in claims 1 and 2 to nucleotides 169 to 539 of RNA 1 of said virus.
5. Method according to claim 1 or 2,
30 characterized in that the fragment has a nucleic acid sequence that corresponds with the homology indicated in claims 1 and 2 to nucleotides 1226 to 1683 of RNA 1 of said virus.
6. Method according to claim 1 or 2
35 characterized in that the fragment has a nucleic acid sequence that corresponds with the homology indicated in

PCT/EP00/00609

Enclosure to letter dated 2 April 2001

claims 1 and 2 to nucleotides 2754 to 3192 of RNA 1 of said virus.

7. Method according to claim 1 or 2 characterized in that the fragment consists of 6746 5 nucleotides.

8. Method as claimed in claims 1-7 characterized in that the fragment is introduced into the cell by means of a DNA vector harboring the fragment and transcription and translation regulatory sequences 10 operably linked therewith.

9. Transformation vector for conveying resistance to BNYVV to a plant, harboring a fragment of at least 15 nucleotides in a sequence that is at least 70% homologous to the corresponding nucleotide sequence 15 of the genomic RNA 1 of said virus, and transcription and translation regulatory sequences operably linked therewith.

10. Vector as claimed in claim 9, wherein the fragment is at least 80%, preferably at least 90%, more 20 preferably at least 95% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of said virus.

11. Vector according to claim 9 or 10, characterized in that the fragment has a nucleic acid sequence that corresponds with the homology indicated in 25 claims 9 and 10 to nucleotides 153 to 3258 of RNA 1 of said virus.

12. Vector according to claim 9 or 10, characterized in that the fragment has a nucleic acid sequence that corresponds with the homology indicated in 30 claims 9 and 10 to nucleotides 169 to 539 of RNA 1 of said virus.

13. Vector according to claim 9 or 10, characterized in that the fragment has a nucleic acid sequence that corresponds with the homology indicated in 35 claims 9 and 10 to nucleotides 1226 to 1683 of RNA 1 of said virus.

14. Vector according to claim 9 or 10, characterized in that the fragment has a nucleic acid

PCT/EP00/00609

Enclosure to letter dated 2 April 2001

sequence that corresponds with the homology indicated in claims 9 and 10 to nucleotides 2754 to 3192 of RNA 1 of said virus.

15. Vector according to claim 9 or 10,
5 characterized in that the fragment consists of 6746 nucleotides.

16. Use of a vector as claimed in claims 9-15 for the transformation of a plant cell.

17. Plant cell, exhibiting a resistance to
10 BNYVV, comprising in its genome a DNA fragment of at least 15 nucleotides in a sequence which is at least 70% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of said virus.

18. Plant cell as claimed in claim 17, wherein
15 the fragment is at least 80%, preferably at least 90%, more preferably at least 95% homologous to the corresponding nucleotide sequence of the genomic RNA 1 of said virus.

19. Plant cell according to claim 17 or 18,
20 characterized in that the fragment has a nucleic acid sequence that corresponds with the homology indicated in claims 17 and 18 to nucleotides 153 to 3258 of RNA 1 of said virus.

20. Plant cell according to claim 17 or 18,
25 characterized in that the fragment has a nucleic acid sequence that corresponds with the homology indicated in claims 17 and 18 to nucleotides 169 to 539 of RNA 1 of said virus.

21. Plant cell according to claim 17 or 18,
30 characterized in that the fragment has a nucleic acid sequence that corresponds with the homology indicated in claims 17 and 18 to nucleotides 1226 to 1683 of RNA 1 of said virus.

22. Plant cell according to claim 17 or 18,
35 characterized in that the fragment has a nucleic acid sequence that corresponds with the homology indicated in claims 17 and 18 to nucleotides 2754 to 3192 of RNA 1 of said virus.

PCT/EP00/00609
Enclosure to letter dated 2 April 2001

20
4

23. Plant cell according to claim 17 or 18, characterized in that the fragment consists of 6746 nucleotides.
24. Plant cell as claimed in claims 17-23 being
5 part of a sugar beet plant that is resistant against BNYVV.
25. Use of a plant cell as claimed in claims 17-23 for the regeneration therefrom of a sugar beet plant that is resistant against BNYVV.
- 10 26. Sugar beet plant, exhibiting a resistance to BNYVV, consisting at least partly of plant cells as claimed in claims 17-23.
27. Progeny of a sugar beet plant as claimed in claim 26.
- 15 28. Seeds of a sugar beet plant as claimed in claim 26.
29. Vegetatively reproducible structures, such as calluses, buds, embryos, from a plant according to claim 26 or progeny according to claim 27.

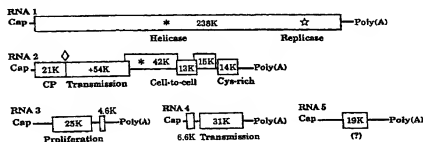
PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12N 15/82, 15/40, 5/10, A01H 5/00		A1	(11) International Publication Number: WO 00/44915
		(43) International Publication Date: 3 August 2000 (03.08.00)	
(21) International Application Number: PCT/EP00/00609		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 26 January 2000 (26.01.00)			
(30) Priority Data: 99200236.0 27 January 1999 (27.01.99) EP			
(71) Applicant (for all designated States except US): SES EUROPE N.V./S.A. [BE/BE]; Industrierpark, Soldatenplein Z 2, No. 15, B-3300 Tienen (BE).			
(72) Inventors; and (75) Inventors/Applicants (for US only): RICHARDS, Kenneth [FR/FR]; 2A, rue Principle, F-67370 Pfulgriesheim (FR). JONARD, Gérard [FR/FR]; 9, quai Chanione Winterer, F-67000 Strasbourg (FR). GUILLEY, Hubert [FR/FR]; 32, rue Herbe, F-67370 Berstett (FR). VAN DUN, Cornelis, Maria, Petrus [NL/NL]; 40, Faunaberg, 4708 CC Roosendaal (NL).			
(74) Agent: VAN SOMEREN, Petronella, Francisca, Hendrika, Maria; Arnold & Siedsma, Sweelinkplein 1, NL-2517 GK The Hague (NL).		Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	

(54) Title: METHOD FOR CONVEYING BNYVV RESISTANCE TO SUGAR BEET PLANTS

BNYVV

(57) Abstract

The present invention relates to a method for conveying resistance to beet necrotic yellow vein virus (BNYVV) to a sugar beet plant, which method comprises the following steps: (a) preparing a DNA fragment of at least 15 nucleotides in a sequence that is essentially homologous to the corresponding nucleotide sequence of the genomic RNA 1 of the beet necrotic yellow vein virus (BNYVV), (b) introducing said DNA fragment, operatively linked to a promoter that is active in sugar beet plants, into a sugar beet plant cell to obtain a transformed sugar beet cell; and (c) regenerating a transgenic sugar beet plant from the transformed sugar beet plant cell. In addition, the invention relates to a DNA vector harboring a fragment of a least 15 nucleotides in a sequence that is essentially homologous to the corresponding nucleotide sequence of the genomic RNA 1 of BNYVV and transcription and translation regulatory sequences operably linked therewith, as well as to the use of said DNA vector for the transformation of a plant cell. The invention further relates to a plant cell comprising in its genome a DNA fragment of at least 15 nucleotides in a sequence that is essentially homologous to the corresponding nucleotide sequence of the genomic RNA 1 of BNYVV and the use of said plant cell for the regeneration thereof of a sugar beet plant that is resistant against BNYVV.

1/7

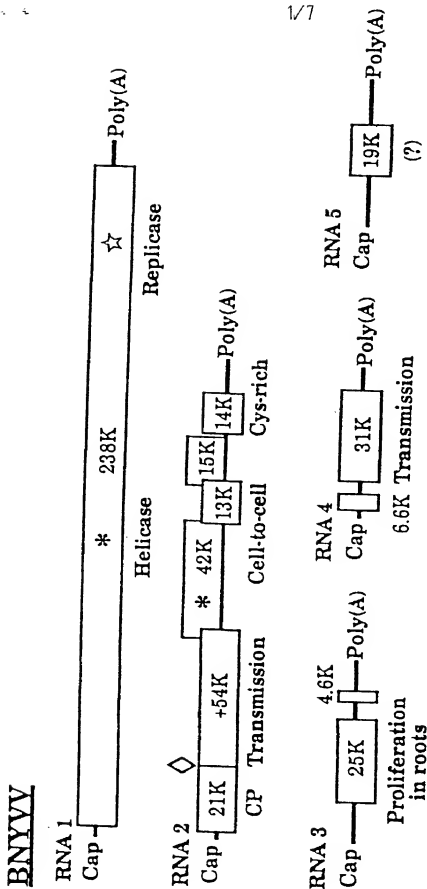


FIG. 1

2/7

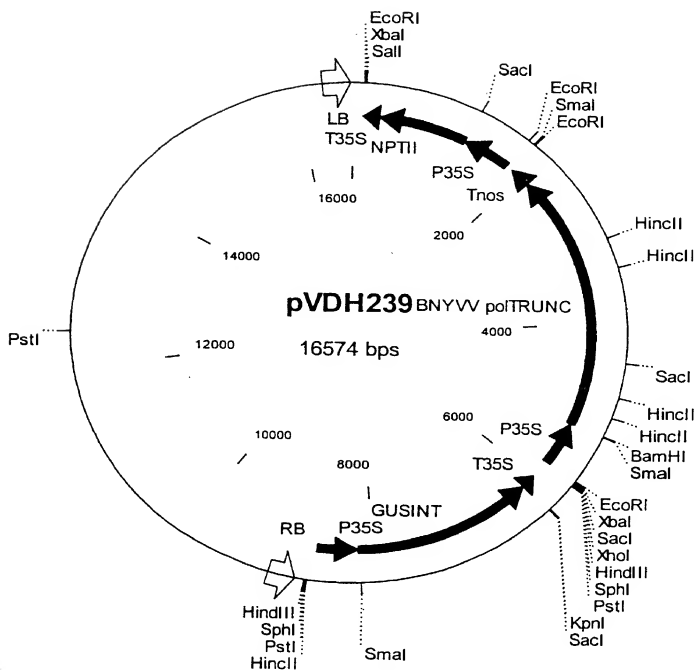


FIG. 2A

3/7

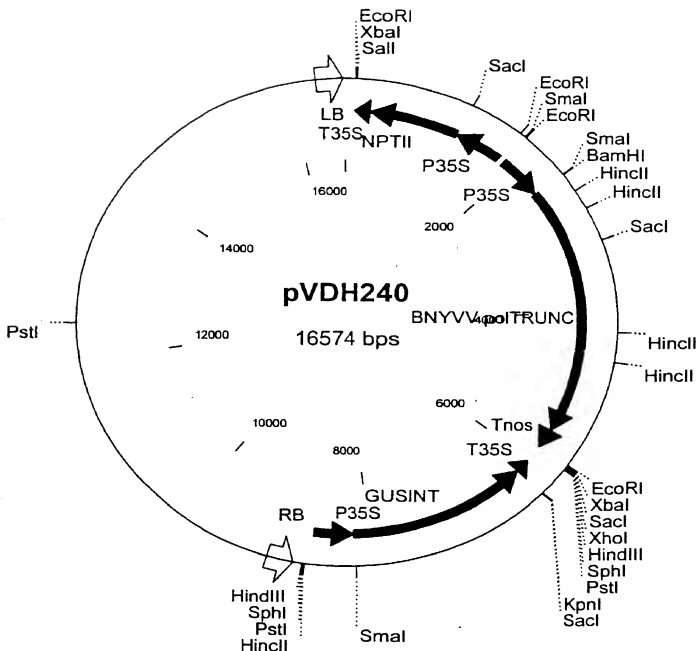


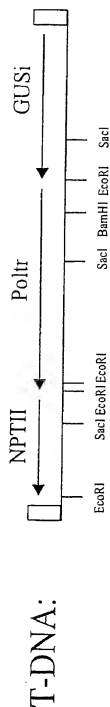
FIG. 2B

Southern analysis sugar beet transformant T157-01 (pVDH239)

WO 00/44915

PCT/EP00/00609

4/7



Probe: GUSi

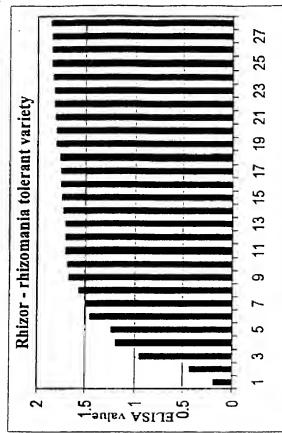
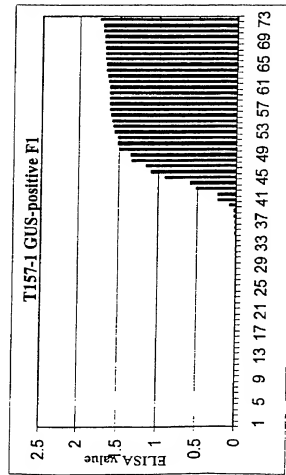
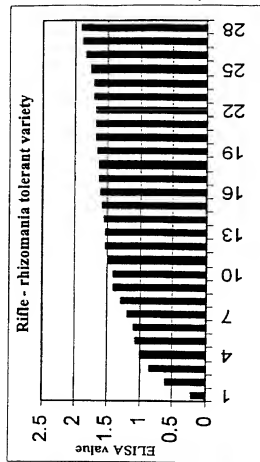
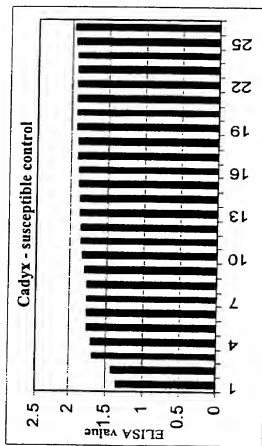


Conclusion: T157-01 contains 3 inserts

FIG. 3

Bioassay rhizomania resistance T157-1

FIG. 4



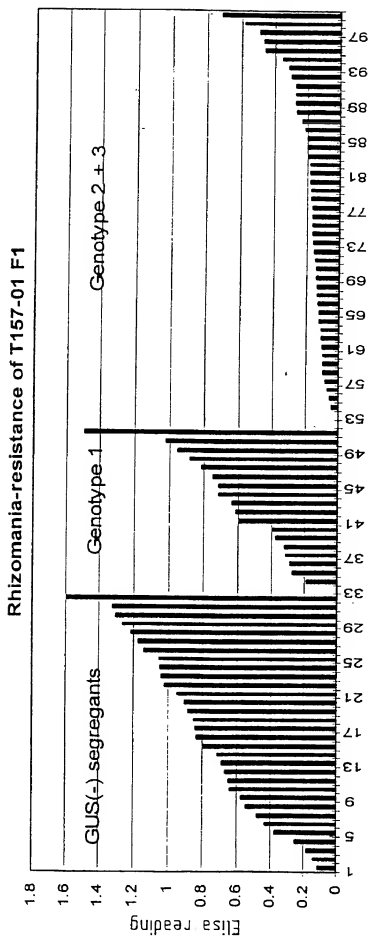
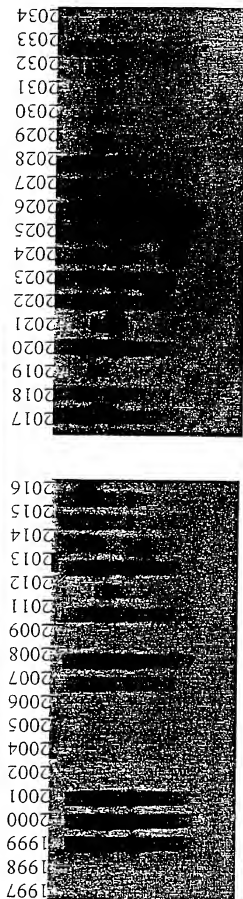


FIG. 5A

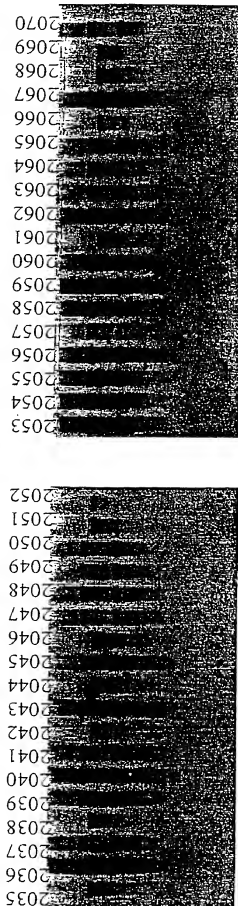
Rhizomania-resistance in T157-01 F1 progeny



097889938

7/7

PCT/EP00/00609



Probe: Gus
Enzyme SacI

FIG. 5B

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Method for conveying BNYVV resistance to sugar beet plants

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on January 26, 2000 as PCT application ~~XXXX~~

~~Application Serial~~ No. PCT/EP00/00609

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

Priority Claimed

99200236.0

Europe

January 27, 1999

☒

☐

(Number)

(Country)

(Day/Month/Year Filed)

Yes

No

(Number)

(Country)

(Day/Month/Year Filed)

☐

☐

Yes

No

(Number)

(Country)

(Day/Month/Year Filed)

☐

☐

Yes

No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status)
		(patented, pending, abandoned)

(Application Serial No.)	(Filing Date)	(Status)
		(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (list name and registration number)

William H. Logsdon	22,132	Paul M. Reznick	33,059	Jesse A. Hirshman	40,016
Russell D. Orkin	25,363	John W. McIlvaine	34,219	James G. Porcelli	33,757
David C. Hanson	23,024	Michael I. Shamos	30,424	Kent E. Baldauf, Jr.	36,082
Richard L. Byrne	28,498	Blynn L. Shideler	35,034	Christian Schuster	43,908
Frederick B. Ziesenheim	19,438	Julie W. Meder	36,216	Dean E. Geibel	42,570
Kent E. Baldauf	25,826	Lester N. Fortney	38,141	Thomas J. Clinton	40,561
Barbara E. Johnson	31,198	Randall A. Notzen	36,882	Nathan J. Prepelka	43,076

Send Correspondence to:

Barbara E. Johnson, 700 Koppers Building, 436 Seventh Avenue, Pittsburgh PA 15219-1818

Direct Telephone calls to: (name and telephone number) **Barbara E. Johnson (412) 471-8815**

Full name of sole or first inventor

RICHARDS, Kenneth

Inventor's signature

Date

12.10.2001

Residence

Pfulgriesheim, France

Citizenship

France

Post Office Address

2A, rue Principle, F-67370 Pfulgriesheim, France

Full name of second joint inventor, if any

JONARD, Gérard

Second inventor's signature

Date

12.10.2001

Residence

Strasbourg, France

Citizenship

France

Post Office Address

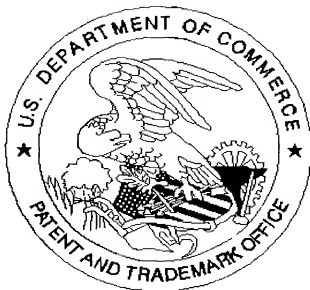
9, quai Chanione Winterer, F-67000 Strasbourg, France

(Supply similar information and signature for third and subsequent joint inventors.)

3-CD	
Full name of third joint inventor, if any	
<u>GUILLEY, Hubert</u>	
Third Inventor's signature	Date
<i>H. Guilley</i>	12.10.2001
Residence	
<u>Berstett, France</u> <i>JCE</i>	
Citizenship	
<u>France</u>	
Post Office Address	
<u>32, rue Herbe, F-67370 Berstett, France</u>	
Full name of fourth joint inventor, if any	
<u>VAN DUN, Cornelis Maria Petrus</u>	
Fourth Inventor's signature	Date
<i>C. Van Dun</i>	07 11 2001
Residence	
<u>Roosendaal, The Netherlands</u> <i>RLX</i>	
Citizenship	
<u>The Netherlands</u>	
Post Office Address	
<u>40, Faunaberg, NL-4708 CC Roosendaal, The Netherlands</u>	
Full name of fifth joint inventor, if any	
Fifth Inventor's signature	Date
Residence	
Citizenship	
Post Office Address	
Full name of sixth joint inventor, if any	
Sixth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	

(Supply similar information and signature for subsequent joint inventors.)

United States Patent & Trademark Office
Office of Initial Patent Examination -- Scanning Division



Application deficiencies found during scanning:

☐ Page(s) _____ of _____ were not present
for scanning. (Document title)

☐ Page(s) _____ of _____ were not present
for scanning. (Document title)

☐ **Scanned copy is best available.**

Some drawing figures are too dark.